Safety and immunogenicity of an Ad26.ZEBOV booster dose in children previously vaccinated with the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen: an open-label, non-randomised, phase 2 trial

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For the French translation of the abstract see Online for appendix 1

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Summary

Background Children account for a substantial proportion of cases and deaths during Ebola virus disease outbreaks. We aimed to evaluate the safety and immunogenicity of a booster dose of the Ad26.ZEBOV vaccine in children who had been vaccinated with a two-dose regimen comprising Ad26.ZEBOV as dose one and MVA-BN-Filo as dose two.

Methods We conducted an open-label, non-randomised, phase 2 trial at one clinic in Kambia Town, Sierra Leone. Healthy children, excluding pregnant or breastfeeding girls, who had received the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in a previous study, and were aged 1–11 years at the time of their first vaccine dose, received an intramuscular injection of Ad26.ZEBOV (5×10^{10} viral particles) and were followed up for 28 days. Primary outcomes were safety (measured by adverse events) and immunogenicity (measured by Ebola virus glycoprotein-specific IgG binding antibody geometric mean concentration) of the booster vaccine dose. Safety was assessed in all participants who received the booster vaccination; immunogenicity was assessed in all participants who received the booster vaccination, had at least one evaluable sample after the booster, and had no major protocol deviations that could have influenced the immune response. This trial is registered with ClinicalTrials.gov, NCT04711356.

Findings Between July 8 and Aug 18, 2021, 58 children were assessed for eligibility and 50 (27 aged 4–7 years and 23 aged 9–15 years) were enrolled and received an Ad26.ZEBOV booster vaccination, more than 3 years after receiving dose one of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen. The booster was well tolerated. The most common solicited local adverse event during the 7 days after vaccination was injection site pain, reported in 18 (36%, 95% CI 23–51) of 50 participants. The most common solicited systemic adverse event during the 7 days after vaccination was headache, reported in 11 (22%, 12–36) of 50 participants. Malaria was the most common unsolicited adverse event during the 28 days after vaccination, reported in 25 (50%, 36–64) of 50 participants. No serious adverse events were observed during the study period. 7 days after vaccination, the Ebola virus glycoprotein-specific IgG binding antibody geometric mean concentration was 28 561 ELISA units per mL (95% CI 20 255–40 272), which was 44 times higher than the geometric mean concentration before the booster dose. 21 days after vaccination, the geometric mean concentration reached 64 690 ELISA units per mL (95% CI 48 356–86 541), which was 101 times higher than the geometric mean concentration before the booster dose.

Interpretation A booster dose of Ad26.ZEBOV in children who had received the two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen more than 3 years earlier was well tolerated and induced a rapid and robust increase in binding antibodies against Ebola virus. These findings could inform Ebola vaccination strategies in paediatric populations.

Funding Innovative Medicines Initiative 2 Joint Undertaking.

Introduction

Children account for approximately 20% of Ebola virus disease cases during outbreaks.[1] Ebola virus disease affects children in many ways: young children (aged <5 years) have a more rapid disease progression and a higher risk of dying than adults,[2] and those children who survive Ebola virus disease can have major psychological trauma, having been separated from their parents and family throughout their disease, having lost time from school, and because they are often stigmatised when they return to their community.[3,4] For these reasons, an effective Ebola prevention strategy for

Research in context

Evidence before this study

We searched PubMed on June 30, 2022, using the terms "Ad26.ZEBOV" AND "booster", for articles published since database inception, with no language restrictions. We identified eight citations. After screening the full texts, we identified three studies that reported results on the safety and immunogenicity of a booster dose of Ad26.ZEBOV in previously vaccinated participants, and one study protocol.

An article by Goldstein and colleagues (2022) described results from a randomised, placebo-controlled, phase 1 trial that assessed the safety and immunogenicity of different regimens of the Ad26.ZEBOV and MVA-BN-Filo vaccines in healthy adults from the USA. A subgroup of participants received a booster vaccination 1 year after their first vaccine dose. The study found that an Ad26.ZEBOV booster vaccination was safe and elicited an anamnestic response in all participants.

An article by Ishola and colleagues (2022) reported results from a study conducted in Sierra Leone and had an open-label, non-randomised stage followed by a randomised, double-blind, controlled stage. Healthy adults who received the two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in the open-label stage were offered an Ad26.ZEBOV booster dose 2 years after their first vaccine dose. The study showed that the booster vaccination was well tolerated and induced a strong anamnestic response, as evidenced by a rapid increase in Ebola virus glycoprotein-specific binding antibody concentrations, which were approximately 40 times higher at 7 days after the booster vaccination and approximately 110 times higher at 21 days after the booster vaccination than before the booster.

An article by Barry and colleagues (2021) reported results from a randomised, placebo-controlled trial conducted in Burkina Faso, Cote d'Ivoire, Kenya, and Uganda. An Ad26.ZEBOV booster, in healthy adults 1 year after their first vaccine dose, was well tolerated and induced a rapid and robust increase in Ebola virus glycoprotein-specific binding antibody concentrations. In those who received the same Ebola vaccine regimen as in our study with a 56-day interval between doses, the binding antibody concentrations were approximately 59 times higher at 7 days after the booster vaccination and approximately 121 times higher at 21 days after the booster vaccination than before the booster.

Larivière and colleagues (2021) described the protocol of an open-label, randomised trial to evaluate the immunogenicity and safety of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in health-care providers in the Democratic Republic of the Congo. In this study, participants were to be randomised to receive an Ad26.ZEBOV booster dose at either 1 year or 2 years after their first vaccine dose; the study is ongoing and the results are not available yet.

Added value of this study

To our knowledge, this is the first study to evaluate the safety and immunogenicity of a booster dose of Ad26.ZEBOV in children aged 4–15 years who had received the two-dose Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen more than 3 years earlier. We found that the Ad26.ZEBOV booster was well tolerated by the study participants, with no safety concerns. The booster vaccination elicited a robust anamnestic response, as shown by a rapid increase in Ebola virus glycoprotein-specific IgG binding antibody concentrations, which were approximately 44 times higher at 7 days after the booster vaccination and approximately 101 times higher at 21 days after the booster vaccination than immediately before the booster.

Implications of all the available evidence

To protect people from Ebola virus disease, effective interventions are needed. Three studies have shown that, in adults who have had previous vaccination, an Ad26.ZEBOV booster is safe and able to produce a rapid and robust increase of binding antibodies against Ebola virus. Our study shows that these findings apply to children, with a very similar extent of increase in antibody concentrations after the booster dose. Our results therefore support the strategy of providing vaccination to children with an additional Ad26.ZEBOV booster to be given at the start of an Ebola virus disease outbreak.

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children living in areas at risk of Ebola virus disease outbreaks is crucial.

A heterologous, two-dose vaccine regimen comprising the monovalent, recombinant, replication-incompetent, adenovirus type 26 (Ad26) vector-based vaccine, encoding the Ebola virus glycoprotein of the Mayinga variant (Ad26.ZEBOV) as dose one, and the recombinant, non-replicating, modified vaccinia Ankara (MVA) vector-based vaccine, encoding glycoproteins from the Ebola virus Mayinga variant, Sudan virus Gulu variant, and Marburg virus Musoke variant, and the nucleoprotein from the Tai Forest virus (MVA-BN-Filo) as dose two, administered 56 days apart, is the only vaccine regimen that has received marketing authorisation (under exceptional circumstances) for immunisation of children aged 1 year or older in the EU.[5]

This vaccine regimen, which has previously been shown to provide protection in vaccinated non-human primates against an Ebola virus challenge,[6] had an acceptable safety profile and induced robust humoral immune responses in children participating in two randomised controlled trials, one in Sierra Leone and the other in Burkina Faso, Cote d'Ivoire, Kenya, and Uganda.[7,8]

The trial in Sierra Leone (VAC52150EBL3001, EBOVAC-Salone) was initiated during the 2014–16 Ebola virus disease outbreak in west Africa, with the aim to assess the efficacy of the vaccine regimen in preventing Ebola virus disease; however, it was not able to achieve

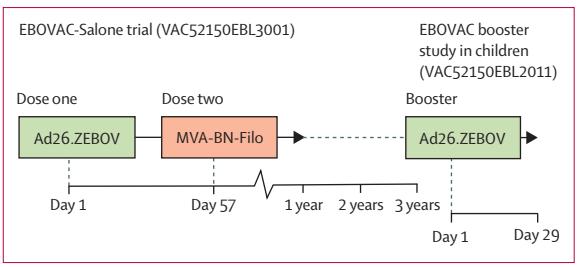

Figure 1: **Study design**
Vaccine doses were 5×10^{10} viral particles for Ad26.ZEBOV (dose one and booster) and 1×10^{8} infectious units for MVA-BN-Filo (dose two).

this objective because the disease incidence declined during the course of the study, as the outbreak was eventually brought under control.[7,9] In the absence of clinical efficacy data, the likelihood of protection induced by the vaccine regimen was inferred by correlating the magnitude of vaccine-elicited immune responses associated with protection in non-human primates with those observed in vaccinated human participants, a statistical approach referred to as immunobridging.[10]

See Online for appendix 2

In previous trials, robust immune responses were observed after dose two of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in both adults and children, but they were also shown to wane over time.[7–9,11–15] In children, the long-term persistence of an immune response beyond 1 year was not known.[7] Although expected, it was also not known whether the vaccine regimen was able to produce immune memory that could be rapidly reactivated by a vaccine booster in children, as had been observed in adults.[9,14,16]

We aimed to evaluate the safety and immunogenicity of a booster dose of the Ad26.ZEBOV vaccine given more than 3 years after the first dose in children who had been vaccinated with the two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen.

Methods

Study design

We conducted an open-label, non-randomised, phase 2 trial (VAC52150EBL2011) at one clinic in Kambia Town, located in Kambia District in the North West Province of Sierra Leone. The study was approved by the Sierra Leone Ethics and Scientific Review Committee, the Pharmacy Board of Sierra Leone, and the London School of Hygiene & Tropical Medicine Ethics Committee. The protocol is available in appendix 2 (pp 7–57).

Participants

Eligible participants were healthy children who had received the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen at least 2 years earlier in the previous EBOVAC-Salone trial (in Kambia District, Sierra Leone; NCT02509494; figure 1) and who were aged 1–11 years at the time of their first vaccine dose in the earlier trial.[7,9] Participants were enrolled in two cohorts by age at the time of their first vaccine dose in the EBOVAC-Salone trial (1–3 years and 4–11 years), and we planned to enrol approximately equal numbers from each of these two age cohorts. Eligible participants were required to be healthy in the investigator's clinical judgement on the basis of medical history, physical examination, vital signs, and a haematological assessment at screening. Adolescent girls who had started their menstrual periods or were aged 12 years or older were required to have a negative urine β-human chorionic gonadotrophin pregnancy test at screening and immediately before booster vaccination. Exclusion criteria included breastfeeding or pregnancy; previous vaccination with a live-attenuated vaccine within 30 days before booster vaccination, or an inactivated vaccine within 15 days before booster vaccination; and previous severe adverse reaction to a vaccine. Eligiblity criteria are listed in full in appendix 2 (pp 25–27). Community engagement was conducted before commencement of the trial to ensure that effective recruitment strategies were in place. Documented informed consent from a community leader was obtained before the start of the study. Parents or guardians of eligible participants were given information about the trial in a language that they understood and they provided written informed consent after passing a test of understanding. Children aged 7 years or older were asked to provide written assent. If the parent or guardian could not read or write, the study procedures were explained by a study team member in a language that the parent or guardian understood, and informed consent was witnessed by a literate third person not involved in the study.

Procedures

All participants received a booster dose of Ad26.ZEBOV (Janssen Vaccines and Prevention, Dessau-Rosslau, Germany). The booster vaccine was administered as a single 0·5 mL intramuscular injection into the deltoid muscle at a dose of 5×10^{10} viral particles.

To record any immediate adverse events, participants were observed for at least 30 min after vaccination. During the first 7 days following booster vaccination, trained field workers visited participants at home to record local and systemic solicited adverse events (defined as signs and symptoms that participants' parents or guardians were specifically asked to report) using a diary card. A haematology panel (haemoglobin, white blood cell count with three-part differential, and platelet count) was performed at 7 days and 21 days after booster vaccination. Parents or guardians of participants received a 24-h telephone number to contact in case of a medical problem. Unsolicited adverse events (defined as events that were reported by the participants or their parents or guardians on their initiative or when they were asked about any symptoms or health problems after vaccination) were recorded from the booster vaccination until the end of the study at 28 days after booster

vaccination. Grade 3 adverse events were defined as severe adverse events that required medical attention but were not immediately life-threatening.

Blood samples for immunogenicity analysis were collected immediately before the booster vaccination and at 7 days and 21 days after the booster vaccination. IgG responses against Ebola virus glycoprotein were analysed using the validated Ebola virus glycoprotein (Kikwit) Filovirus Animal Non-Clinical Group ELISA, as in previous studies.[7–9,11–15] The test has a lower limit of quantification of 36·11 ELISA units per mL and an upper limit of quantification of 194 938·88 ELISA units per mL. The analysis was conducted at Q² Solutions, San Juan Capistrano, CA, USA.

There were no major protocol deviations during the conduct of the study. There were two minor deviations. One was the use of infrared temperature scanning machines instead of using axillary temperature thermometers in all 50 participants. Infrared temperature scanning machines were considered to be more acceptable by study participants and staff during the COVID-19 outbreak. The other minor protocol deviation was a missed day 22 visit in one participant in the age 4–11 years cohort. These two deviations were not considered to have the potential to affect the safety of participants or to influence the immune response.

Outcomes

The primary outcomes were the safety and tolerability of the Ad26.ZEBOV booster vaccination, measured as the number of participants with solicited local and systemic adverse events in the 7 days after vaccination and unsolicited adverse events, including serious adverse events, in the 28 days after vaccination; and the vaccine-induced humoral immune response to the Ebola virus glycoprotein at 7 days and 21 days after vaccination, measured by Ebola virus glycoprotein-specific IgG binding antibody geometric mean concentration.

A planned exploratory outcome was the neutralising antibody response against the Ad26 vector before booster vaccination, as measured by a virus neutralisation assay, but this analysis had not yet been completed and is not reported in this manuscript. Results for this exploratory outcome will be made available on the trial registration page on ClinicalTrials.gov.

Statistical analysis

The study sample size (n=50) was a convenience sample and was not based on formal hypothesis testing considerations. However, using the sample size formula for estimating a population proportion with a given absolute precision, $n=Z^2\times P(1-P)/d^2$,[17] we calculated that this sample size would have allowed an estimation of the proportion of participants with solicited or unsolicited adverse events after booster vaccination with a plus or minus 10% margin of error (ie, an absolute precision within 10 percentage points of an anticipated proportion, with 95% confidence), assuming that approximately 15% of participants had a solicited adverse event or an unsolicited adverse event: $1{\cdot}96^2\times(0{\cdot}15\times0{\cdot}85)/0{\cdot}1^2=49$. A sample size of 50 participants (approximately 25 in

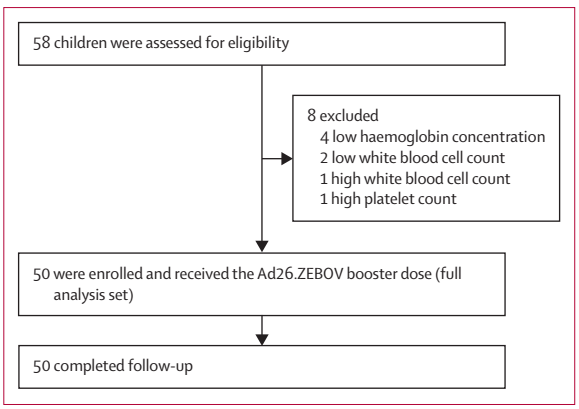

Figure 2: **Study profile**

	Age 1–3 years cohort (n=27)	Age 4–11 years cohort (n=23)
Age, years		
Median (IQR)	5 (4–5)	13 (11–14)
Range	4–7	9–15
Weight-for-age percentile*		
Number assessed	26	5
Median (IQR)	32% (15–53)	52% (7–65)
Lower than 2nd percentile	2 (8%)	0
Height-for-age percentile*		
Number assessed	26	6
Median (IQR)	42% (12–58)	61% (12–67)
Lower than 2nd percentile	1 (4%)	0
Weight-for-height percentile†		
Number assessed	21	..
Median (IQR)	38% (21–59)	..
Lower than 2nd percentile	2 (10%)	..
BMI, kg/m²‡		
Number assessed	..	17
Median (IQR)	..	22 (6–59)
Lower than 2nd percentile	..	1 (6%)
Sex		
Male	19 (70%)	12 (52%)
Female	8 (30%)	11 (48%)
Duration since first vaccine dose in the EBOVAC-Salone trial, years		
Median (IQR)	3·11 (3·08–3·13)	3·83 (3·82–3·85)
Range	3·04–3·23	3·53–3·93

Data are n or n (%) unless otherwise stated. *Calculated in children aged 11 years or younger (at enrolment in the current study) according to WHO growth charts. †Calculated in children aged 5 years or younger (at enrolment in the current study) according to WHO growth charts. ‡BMI was calculated for older children only (age 12–17 years at enrolment in the current study).

Table 1: **Baseline characteristics at the booster study screening visit, by age cohort at first vaccine dose in the EBOVAC-Salone trial**

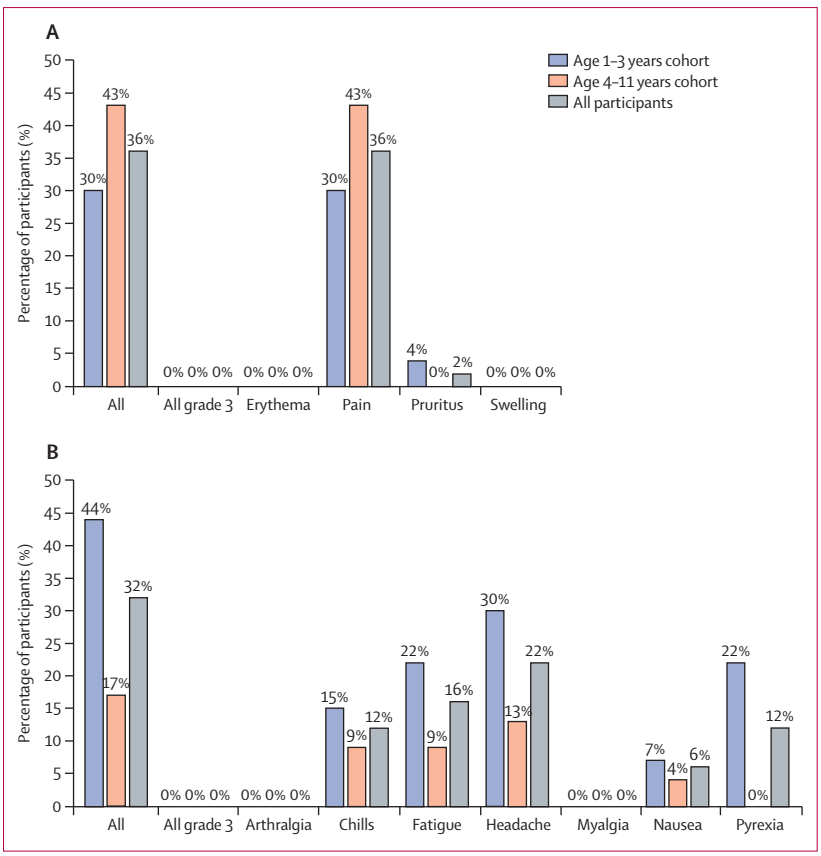

Figure 3: **Solicited adverse events after the Ad26.ZEBOV booster vaccination**
(A) Solicited local adverse events. (B) Solicited systemic adverse events. Solicited adverse events were observed during the period of 7 days after Ad26.ZEBOV booster vaccination.

each of the two age cohorts) would also allow for adequate characterisation of the humoral immune response after booster vaccination.

The primary analysis was performed when all participants had completed the study. The primary analysis set for safety (full analysis set) comprised all participants who received the booster vaccine. The primary analysis set for immunogenicity (per-protocol set) included all participants who received the booster vaccine, had at least one evaluable immunogenicity serum sample after vaccination, and had no major protocol deviations considered to have an effect on the immune response to the booster vaccination.

We merged the database containing the immunogenicity data from this booster study with the database containing the immunogenicity data from the EBOVAC-Salone trial, in particular the antibody concentration before the first dose (Ad26.ZEBOV) measured in the same participants using the same assay in the same laboratory. Thus, we were able to calculate the percentage of participants with an immunogenic response in the booster study with respect to the baseline before the first dose in the EBOVAC-Salone trial. Participants were considered to have a response by ELISA if samples were negative at baseline before the first dose and positive at following evaluations with a value that was greater than 2·5 times the lower limit of quantification of 36·11 ELISA units per mL, or if a sample was positive both at baseline before the first dose and at following evaluations with a greater than 2·5-times increase from baseline. The definition of response is the same as that used in the previous EBOVAC-Salone trial.[7,9] Binding antibody responses against Ebola virus glycoprotein were summarised as geometric mean concentrations. For this calculation, all values of less than the lower limit of quantification were imputed with half the lower limit of quantification value. CIs were calculated using the Clopper-Pearson methods for percentages and using linear regression for geometric mean concentrations. We present two-sided 95% CIs for all safety and immunogenicity point estimates that were not 0, except when responder rates were 100%; in this case, we present one-sided 97·5% CIs.

We did a post-hoc analysis to compare antibody concentrations at 7 and 21 days after booster vaccination between participants classified as responders and non-responders at day 1 before the booster administration. For this analysis, geometric mean ratios were used to compare geometric mean concentrations, and p values were calculated using a *t* test.

Stata 16 was used for the statistical analyses. This study is registered with ClinicalTrials.gov, NCT04711356.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between July 8 and Aug 18, 2021, 58 children were assessed for eligibility and 50 were enrolled and received an Ad26.ZEBOV booster vaccination more than 3 years after their first vaccine dose, 27 (54%) of whom were in the original age 1–3 years parent study cohort (aged 4–7 years at the time of screening for this booster study), and 23 (46%) of whom were in the original age 4–11 years parent study cohort (aged 9–15 years at the time of screening for this booster study). Follow-up was completed on Sept 17, 2021. The safety analysis included all 50 study participants (figure 2). Baseline characteristics of the participants are shown in table 1.

Solicited adverse events were all mild (grade 1) and of short duration (≤3 days; figure 3; appendix 2 p 2). At least one solicited local adverse event was reported by 18 (36%, 95% CI 23–51) of 50 participants after booster vaccination: eight (30%, 14–50) of 27 in the age 1–3 years cohort and ten (43%, 23–66) of 23 in the age 4–11 years cohort. All 18 participants who reported at least one solicited local adverse event reported injection site pain and one (2%) participant also reported pruritus at the injection site (figure 3A; appendix 2 p 2). 16 (32%, 95% CI 20–47) of

50 participants reported at least one solicited systemic adverse event after booster vaccination (figure 3B; appendix 2 p 2): 12 (44%, 25–65) of 27 in the age 1–3 years cohort and four (17%, 5–39) of 23 in the age 4–11 years cohort. Headache was the most frequently reported solicited systemic adverse event, followed by fatigue, chills, and pyrexia (figure 3B; appendix 2 p 2). The most frequent unsolicited adverse event after booster vaccination was malaria, reported by 25 (50%, 95% CI 36–64) of 50 participants: 19 (70%, 50–86) of 27 in the age 1–3 years cohort and six (26%, 10–48) of 23 in the age 4–11 years cohort (appendix 2 p 3). No grade 3 adverse events and no serious adverse events were reported throughout the study (appendix 2 p 3).

After booster vaccination, the most commonly reported laboratory abnormalities were low haemoglobin concentration and low white blood cell count (appendix 2 pp 4–5). Two (4%, 95% CI 0–14) of 50 participants had haemoglobin concentrations of less than the local normal laboratory range at 7 days after the booster vaccination and three (6%, 1–17) of 49 had haemoglobin concentrations of less than the local normal laboratory range at 21 days after the booster. One (2%, 95% CI 0–11) of 50 participants had low white blood cell count at 7 days after the booster and two (4%, 0–14) of 49 had low white blood cell count at 21 days after the booster. None of these abnormalities were considered clinically relevant by the investigator. One participant in the youngest age cohort had a low platelet count ($80·0 \times 10^9$ cells per L) at 21 days after the booster, which was considered clinically relevant and was reported as an adverse event (appendix 2 pp 3, 5). The participant was asymptomatic and a repeated haematology assessment 10 days later showed a normal platelet count ($150·0 \times 10^9$ cells per L).

All 50 participants in the study fulfilled the criteria for the per-protocol analysis set for immunogenicity and the results of this analysis are presented in table 2 and figure 4.

Before the booster vaccination, participants' geometric mean concentration of binding antibodies against the Ebola virus glycoprotein was 640 ELISA units per mL (95% CI 461–888) overall, 934 ELISA units per mL (568–1534) in the age 1–3 years cohort, and 418 ELISA units per mL (287–608) in the age 4–11 years cohort (table 2). When compared with the binding antibody geometric mean concentration at baseline before their first vaccine dose, 40 (87%, 95% CI 74–95) of 46 participants still had a response at a median of 3·2 years from the time of dose one vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in the EBOVAC-Salone trial. In the age 1–3 years cohort, 23 (96%, 95% CI 79–100) of 24 participants still had a response at a median of 3·1 years from the time of dose one vaccination in the EBOVAC-Salone trial. In the age 4–11 years cohort, 17 (77%, 95% CI 55–92) of 22 participants still had a response at a median of 3·8 years from the time of dose one vaccination.

	Age 1–3 years cohort (n=27)	Age 4–11 years cohort (n=23)	Overall (n=50)
Day 1 (baseline before booster vaccine)			
Number assessed	26	23	49
Geometric mean concentration, ELISA units per mL (95% CI)	934 (568–1534)	418 (287–608)	640 (461–888)
Participants with response*	23/24 (96%, 79–100)	17/22 (77%, 55–92)	40/46 (87%, 74–95)
Day 8 (7 days after booster vaccine)			
Number assessed	27	23	50
Geometric mean concentration, ELISA units per mL (95% CI)	30 463 (18 087–51 307)	26 478 (16 512–42 461)	28 561 (20 255–40 272)
Participants with response*	25/25 (100%, 86–100)	22/22 (100%, 85–100)	47/47 (100%, 92–100)
Day 22 (21 days after booster vaccine)			
Number assessed	27	22	49
Geometric mean concentration, ELISA units per mL (95% CI)	71 143 (47 819–105 844)	57 564 (36 375–91 095)	64 690 (48 356–86 541)
Participants with response*	25/25 (100%, 86–100)	22/22 (100%, 85–100)	47/47 (100%, 92–100)

Data are n unless otherwise stated. *Expressed as n/N (%, two-sided 95% CI or, when 100%, one-sided 97·5% CI), where n is the number of participants with response at that timepoint and N is the total number of participants with baseline data at first vaccine dose in the EBOVAC-Salone trial and at that timepoint. Participants were considered as having a response by ELISA if samples were negative at baseline before the first vaccine dose and positive at following evaluations with a value that was greater than 2·5 times the lower limit of quantification (36·11 ELISA units per mL), or if a sample was positive both at baseline before the first vaccine dose and at following evaluations and there was a greater than 2·5-times increase from baseline.

Table 2: **Ebola glycoprotein-specific binding antibody concentrations by age cohort at first vaccine dose in the EBOVAC-Salone trial and overall**

7 days after the booster vaccination, participants' Ebola virus glycoprotein binding antibody geometric mean concentration increased to 28 561 ELISA units per mL (95% CI 20 255–40 272) overall, 30 463 ELISA units per mL (18 087–51 307) in the age 1–3 years cohort, and 26 478 ELISA units per mL (16 512–42 461) in the age 4–11 years cohort (table 2).

21 days after the booster vaccination, participants' Ebola virus glycoprotein binding antibody geometric mean concentration increased to 64 690 ELISA units per mL (95% CI 48 356–86 541) overall, 71 143 ELISA units per mL (47 819–105 844) in the age 1–3 years cohort, and 57 564 ELISA units per mL (36 375–91 095) in the age 4–11 years cohort (table 2).

When compared with the binding antibody geometric mean concentration before dose one vaccination, 47 (100%, one-sided 97·5% CI 92–100) of 47 participants with available data had a response at both 7 days and 21 days after the booster vaccination (table 2).

The overall binding antibody geometric mean concentration at 7 days after the booster vaccination was approximately 44 times higher than the geometric mean concentration before the booster; 32 times higher in the age 1–3 years cohort and 63 times higher in the age 4–11 years cohort. The overall binding antibody geometric

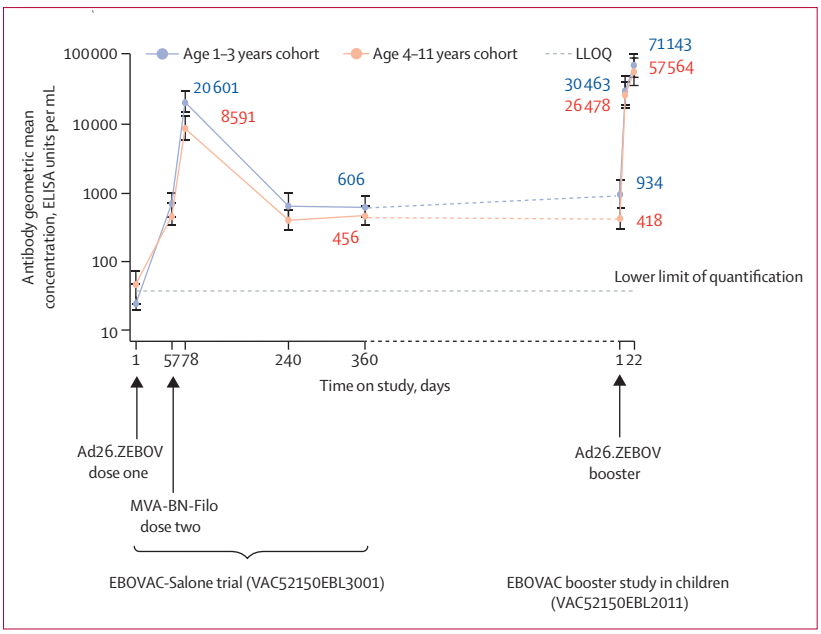

Figure 4: **Ebola virus glycoprotein-specific binding antibody concentrations**
The response profile of each age group is shown as geometric mean concentrations of anti-Ebola virus glycoprotein IgG. Error bars show 95% CIs. Labels for day 8 (7 days after the booster vaccination) and day 29 (28 days after the booster vaccination) in the VAC52150EBL2011 study have been omitted.

mean concentration at 21 days after the booster vaccination was approximately 101 times higher than the geometric mean concentration before the booster; 76 times higher in the age 1–3 years cohort and 137 times higher in the age 4–11 years cohort. The comparison of antibody concentrations between responders and non-responders at day 1 after booster vaccination, performed as a post-hoc analysis, showed that responders had higher binding antibody geometric mean concentrations at 21 days after the booster than non-responders (geometric mean ratio 2·39, 95% CI 1·21–4·74; p=0·014; appendix 2 p 6).

Discussion

This is the first clinical study of an Ad26.ZEBOV booster vaccination in children who had previously been vaccinated with the two-dose Ad26.ZEBOV and MVA-BN-Filo vaccine regimen. The booster vaccination was well tolerated, with injection site pain being the most frequent solicited local adverse event, and headache being the most frequent solicited systemic adverse event, followed by fatigue, chills, and pyrexia. No serious adverse events were reported in the 28 days after the booster dose. The Ad26.ZEBOV booster vaccine induced Ebola virus glycoprotein-specific binding antibody responses in all participants at 7 days and 21 days after the booster, with 44-times higher antibody concentration at 7 days and 101-times higher antibody concentration at 21 days for both age cohorts combined compared with concentration before the booster dose.

The safety results after the Ad26.ZEBOV booster dose in our study are consistent with the safety profile of the Ad26.ZEBOV dose one in children of similar age in the EBOVAC-Salone trial.[7]

The immunogenicity findings in children in this study are consistent with the data from adults in the EBOVAC-Salone trial, which showed that an Ad26.ZEBOV booster vaccination given 2 years after initial vaccination was well tolerated and induced a robust increase in binding antibody concentrations.[9] An Ad26.ZEBOV booster given to healthy adults 1 year after initial vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen was also shown to be well tolerated and strongly immunogenic in the VAC52150EBL2002 study,[14] which was conducted in Kenya, Burkina Faso, Côte d'Ivoire, and Uganda.

To our knowledge, this is also the first study to collect long-term immunogenicity data in children vaccinated with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen. The median time from receipt of first vaccine dose with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in the EBOVAC-Salone study to baseline assessment before booster vaccination in the current study was 3·2 years. At this timepoint, binding antibodies were still detectable and 87% of all participants were classified as still having a response, indicating that the humoral immune response to the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in children is durable to at least 3 years. When stratified by age group, the median time from the first vaccine dose in the EBOVAC-Salone trial to baseline assessment in the current study was 3·1 years in the age 1–3 years cohort (96% still had a response) and 3·8 years in the age 4–11 years cohort (77% still had a response). In a post-hoc analysis, participants who were responders before the booster dose had higher antibody concentrations at 21 days after the booster vaccine than non-responders. However, our results also show that all non-responders had a response after the booster vaccination, suggesting that the booster was also immunogenic in this group.

This study has some limitations. The follow-up period after booster vaccination was only 28 days, due to the end of the grant that funded this study. Although this did not affect the collection of solicited and unsolicited adverse events after vaccination, which continued up to 7 days for solicited adverse events and 28 days for unsolicited adverse events, as in previous studies, it limited the timeframe for collection of serious adverse events to 28 days after booster vaccination.[9,14] However, in previous studies with longer follow-up periods, none of the serious adverse events reported were considered related to the booster vaccine.[9,14] Therefore, we believe that a 28-day follow-up period was sufficient to characterise the safety of the Ad26.ZEBOV booster dose. In the previous studies, adults who were followed up for 1 year after receiving the Ad26.ZEBOV booster showed binding antibody geometric mean concentrations at this timepoint that were higher than at 1 year after the initial Ad26.ZEBOV and MVA-BN-Filo vaccine regimen administration.[9,14] Because the children in our study showed a binding antibody response

similar to that previously observed in adults at 7 days and 21 days after booster vaccination, it is plausible that their binding antibody kinetics will continue to reflect those of adults at later timepoints, and that the binding antibody geometric mean concentrations will be maintained at levels higher than after the initial Ad26.ZEBOV and MVA-BN-Filo vaccine regimen for at least 1 year after booster vaccine administration. Another limitation of this study is that neutralising antibodies against Ebola virus could not be assessed within the timeframe of the grant that funded the study. However, previous clinical trials and non-human primates' challenge studies have shown that the titres of neutralising antibodies strongly correlated with the concentration of Ebola virus glycoprotein binding antibodies after the initial Ad26.ZEBOV and MVA-BN-Filo vaccine regimen administration, therefore neutralising antibodies are likely to increase similarly to binding antibodies after booster vaccination.[6,7,9,10,15] Binding antibodies were also identified as the immune parameter most highly correlated with non-human primates' survival in challenge studies and were selected for use in the immunobridging analysis.[6,10] The assessment of cellular immune responses after booster vaccination was also not included in the study protocol because the laboratory in Sierra Leone was not capable of processing peripheral blood mononuclear cells at the time when the protocol was written, and we could not have established the technique within the timeframe of the grant. The ability of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen to induce cellular immune responses has been studied previously in adults and children,[8,11–14] but data in children are scarce;[8] therefore, it would be important in future studies to collect further data in children and also assess cellular immune responses after the booster dose in both adults and children.

Finally, an important limitation of the study is that we do not know if the concentrations of binding antibodies observed after booster vaccination indicate protection against Ebola virus disease because an antibody threshold correlating with protection has not yet been established. However, considering that the clinical benefit of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen was inferred using the immunobridging model based on the vaccine-induced binding antibody concentrations, and that these were higher after booster vaccination than after the initial vaccine regimen administration, it is plausible that the booster dose is beneficial in providing an increased likelihood of protection against Ebola virus disease.

This study provides valuable data that can inform future Ebola vaccination strategies in paediatric populations. The 56-day interval Ad26.ZEBOV and MVA-BN-Filo vaccine regimen has received marketing authorisation for immunisation of adults and children aged 1 year or older in the EU, with the possibility of an Ad26.ZEBOV booster in previously vaccinated people at imminent risk of infection with Ebola virus.[18] Our results, which show that the Ad26.ZEBOV booster vaccination induces a strong anamnestic response within 7 days in children vaccinated more than 3 years previously, support this recommendation in paediatric populations. Vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen could be considered for children in areas with Ebola risk, with an additional Ad26.ZEBOV booster provided if there is an imminent risk of exposure to Ebola virus, such as during an Ebola virus disease outbreak. Modelling studies are needed to evaluate the best administration strategy in these emergency situations (ie, ring vaccination *vs* mass vaccination approach). Outside outbreak situations, whether a booster dose is needed after an interval of time from the first vaccination, and the optimal timing for booster administration, still remain to be established. Results from the ongoing VAC52150EBL2007 study will elucidate if there is any difference in the elicited immune response if the booster dose is given either 1 year or 2 years after the first vaccination,[19] while another booster study, VAC52150EBL2010 (NCT05064956), assessing the safety and immunogenicity of a booster dose in previously vaccinated HIV-positive adults, will also provide long-term immunogenicity data after more than 4 years from initial vaccination with the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in this group. Further research is also needed to define the best approach for the administration of the Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in paediatric populations in countries with Ebola risk; for example, whether vaccination should be given through campaigns or integrated within the routine paediatric immunisation schedule.

Contributors

DM drafted the manuscript and conducted the literature search. DM, FB, JF, NEC, BKe, EM-LC, AG, CM, KL, MS, BLo, CR, BLe, BG, and DW-J were involved in the study concept and design, study conduct, and interpretation of results. DW-J was the lead scientist for the programme (EBOVAC1) at the London School of Hygiene & Tropical Medicine. CR was the lead scientist for the programme at Janssen Vaccines and Prevention. BLe was the clinical trial principal investigator in Sierra Leone. AB and ADB were the coordinators of the study in Sierra Leone. ABK, JK, MC, GFD, and HHA contributed to enrolment and clinical care of participants and data collection. YN was responsible for data management. BJL, MTK, GTO, and BLo were responsible for laboratory sample analysis, samples management, and laboratory results interpretation. ATD, AB, and JK were responsible for community engagement activities. BKo and PB were the clinical trial pharmacists and were responsible for study vaccine preparation and dispensing. PA conducted the statistical analysis. PA and DM accessed and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

Janssen Vaccines and Prevention was the vaccine manufacturer and donated the vaccine for this study. BKe, AG, CM, KL, and CR were full-time employees of Janssen, Pharmaceutical Companies of Johnson & Johnson, at the time of the study. AG, CM, KL, and CR report ownership of shares in Janssen, Pharmaceutical Companies of Johnson & Johnson.

All other authors declare funding from the Innovative Medicines Initiative 2 Joint Undertaking.

Data sharing

Following publication of the primary and exploratory objectives as detailed in the protocol, individual deidentified participants' data and a data dictionary will be made available upon request via the London School of Hygiene & Tropical Medicine research data repository, LSHTM Data Compass at http://datacompass.lshtm.ac.uk. Requests with a defined analysis plan can be sent via LSHTM Data Compass. The clinical study protocol is available in appendix 2.

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